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NORMAL VALUES FOR IONIZED CALCIUM IN THE PLASMA OF NORMAL LACTATING EWES AND SUCKLING LAMBS: RELATION TO PLASMA TOTAL CALCIUM, INORGANIC PHOSPHATE, MAGNESIUM AND TOTAL PROTEINS

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OPSOMMING: GEÏONISEERDE KALSIUM IN DIE PLASMA VAN NORMALE LAKTERENDE OOIE EN SUIPLAMMERS: VERWANTSKAP MET PLASMA TOTALE KALSIUM, ANORGANIESE FOSFAAT, MAGNESIUM EN TOTALE PROTEÏENE

Die gemiddelde geïoniseerde plasma kalsium van 35 normale lakterende ooie was 4,25 ±0,49 mg/100 ml (1,06 ±0,12 mM/l) en in 26 normale enkel 21–28 dae oud suiplammers 4,47 ± 0,65 mg/100 ml (1,12 ±0,16 mM/l). In ooie het dit 'n gemiddelde van 41,29 ± 4,44% van die totale kalsium uitgemaak en in lammers 41,69 ±5,69% Alhoewel daar betekenisvolle korrelasie tussen geïoniseerde kalsium en totale kalsium in beide ooie ($P < 0,05$; $r = 0,416$) en lammers ($P < 0,05$; $r = 0,417$) gevind is, was dit nie hoog genoeg om geïoniseerde kalsiumwaardes te voorspel vanaf totale kalsium waardes nie. By ooie is 'n betekenisvolle negatiewe korrelasie tussen totale kalsium en anorganiese fosfaat ($P < 0,05$; $r = -0,388$), 'n hoogs betekenisvolle negatiewe korrelasie tussen geïoniseerde kalsium en anorganiese fosfaat ($P < 0,01$; $r = -0,625$) en 'n hoogs betekenisvolle positiewe korrelasie tussen totale kalsium en magnesium ($P < 0,01$; $r = 0,442$) gevind. Soortgelyke korrelasie is nie in lammers gevind nie. Geen verwantskappe is gevind tussen totale kalsium, geïoniseerde kalsium of die verhouding tussen die twee en plasmaproteïne by ooie of lammers nie.

SUMMARY:

The mean plasma ionized calcium in 35 normal lactating ewes was 4,25 ±0,49 mg/100 ml (1,06 ±0,12 mM/l) and in 26 normal single 21–28 day old suckling lambs was 4,47 ±0,65 mg/100 ml (1,12 ±0,16 mM/l). On average this constituted 41,29 ±4,44% of total calcium in ewes and 41,69 ±5,69% in lambs. Although there was a significant correlation between ionized calcium and total calcium in both ewes ($P < 0,05$; $r = 0,416$) and lambs ($P < 0,05$; $r = 0,417$) this was not of a sufficiently high order to predict ionized calcium from total calcium levels. In ewes there was a significant negative correlation between total calcium and inorganic phosphate ($P < 0,05$; $r = -0,388$), a highly significant negative correlation between ionized calcium and inorganic phosphate ($P < 0,01$; $r = 0,625$) and a highly significant positive correlation between total calcium and magnesium ($P < 0,01$; $r = 0,442$). Similar correlations were not found in lambs. In both ewes and lambs no relationship was found between total calcium, ionized calcium or the ratio between the two and plasma protein.

Because ionized calcium is physiologically the most important fraction of total calcium it was decided to determine its level in the blood of normal animals as part of an extensive study of calcium metabolism in sheep. Values for serum ionized calcium in sheep were first reported by Belonje (1973). These levels were determined on serum from normal ewes which were either non-pregnant or within the first three weeks of pregnancy. In addition it was shown that, although there was a highly significant linear correlation between ionized calcium and total calcium, this was not of a significantly high order to be able to predict ionized calcium values from total calcium values. There were no significant correlations between ionized calcium, or the ratio of ionized to total calcium, or total plasma proteins and plasma magnesium.

The present investigation was undertaken to determine the levels of ionized calcium in the blood of normal lactating ewes and suckling lambs. At the same time plasma total calcium, inorganic phosphate, magnesium and total proteins were determined. The ionized calcium, total calcium and the ratio between the two were then correlated with the other parameters measured.

Procedure

Animals

Twenty five clinically normal lactating ewes and 26 single 21–28 day old lambs, kept on artificial pastures at the Welgevallen experimental farm, were used.

Collection of blood specimens

As a great deal of difficulty had been experienced previously particularly in obtaining serum from small quantities of blood for ionized calcium determinations, the following procedure was adopted:

The animals were handled carefully to avoid excitement and were bled in the standing position. The jugular vein was occluded only momentarily to locate it for puncture and then free-flowing blood was drawn anerobically through the same needle into two plastic heparinized syringes the points of which were sealed with plastic caps and then kept in crushed ice. The first syringe (10 ml capacity containing 10 i.u dry heparin per ml blood) was centrifuged point downwards as soon as possible after sample collection and plasma inorganic phosphate was determined immediately there-

after. The rest of the plasma was kept at −20°C and analysed later for calcium, magnesium and total proteins. The second syringe (1 ml capacity containing 2,5 i.u dry heparin per ml blood) was used for the collection of blood for the determination of ionized calcium. It has previously been shown that whereas the concentrations of heparin which are usually used interfere with ionized calcium determinations a concentration of about 2,5 i.u. per ml blood has no such effect while still inhibiting blood coagulation (Radde, Höffken, Parkinson, Sheepers & Luckham, 1971; Blum, Ramberg, Johnson & Kronfeld, 1972). This capped syringe, filled with blood, was then centrifuged point upwards and the plasma could then be removed through the tip of the syringe into the syringe used in the calcium ion-exchange electrode system.

This system proved to be quick and easy and it ensured that where necessary specimens remained anaerobic. Moreover, the blood was cooled quickly in the crushed ice minimizing changes in the inorganic phosphate fraction which was determined as soon as possible after collecting the blood.

Analytical methods

a. *Ionized calcium:* The same method of processing the samples and bracketing of each determination with a standard was used as described previously (Belonje, 1973). An Orion Model 99–20 Serum Calcium Flow-thru System and Model 801 Digital pH/mV meter was used for the analyses.

b. *Total plasma calcium and magnesium:* Plasma was diluted 1 in 20 by means of an automatic pipette (Micromedic Systems Inc.) with 0,1% lanthanum solution and compared against commercial standards (Hopkin and Williams) also diluted with 0,1% lanthanum on a Techtron Model 1 200 atomic absorption spectrophotometer.

c. *Plasma inorganic phosphate:* This was determined by means of the colorimetric method of Delsal & Manhouri (1958).

d. *Total plasma proteins:* These were determined by means of the colorimetric method of Weichselbaum (1946).

e. *Statistical methods:* As described by Snedecor & Cochran (1967).

Results

The levels of the nine blood constituents in the 35 lactating ewes are presented in Table 1 and the correlations between ionized calcium, the ratio between ionized calcium and total calcium, total calcium and the other blood constituents in Table 2.

Table 1

Levels of six blood constituents in 35 normal lactating ewes

Determination	Mean	SD
Total plasma calcium mg/100 ml	10,29	0,47
Plasma ionized calcium mg/100 ml	4,25	0,49
Ratio ionized : total calcium x 100	41,29	4,44
Plasma inorganic phosphate mg/100 ml	5,30	1,26
Plasma magnesium mg/100 ml	2,23	0,46
Total plasma proteins g/100 ml	6,54	0,37

Table 2

Correlations between ionized calcium, calcium ratio (ionized : total), total calcium and other blood constituents measured in 35 normal lactating ewes

Correlation between –	Correlation coefficient	Significance
Ionized calcium and total calcium	0,416	P < 0,05
Ionized calcium and inorganic phosphate	−0,625	P < 0,01
Ionized calcium and magnesium	−0,140	NS
Ionized calcium and total protein	−0,290	NS
Calcium ratio and inorganic phosphate	−0,530	P < 0,01
Calcium ratio and magnesium	−0,309	NS
Calcium ratio and total protein	−0,264	NS
Total calcium and inorganic phosphate	−0,388	P < 0,05
Total calcium and magnesium	0,442	P < 0,01
Total calcium and total protein	−0,147	NS

Correlation coefficient required for significance:
P < 0,05 = 0,335; P < 0,01 = 0,430 NS = Not significant

Table 3

Levels of six blood constituents in 26 normal 21–28 day old lambs

Determination	Mean	SD
Total plasma calcium mg/100 ml	10,73	0,67
Plasma ionized calcium mg/100 ml	4,47	0,65
Ratio ionized : total calcium x 100	41,69	5,69
Plasma inorganic phosphate mg/100 ml	9,97	1,52
Plasma magnesium mg/100 ml	2,20	0,27
Total plasma proteins g/100 ml	5,46	0,20

Table 4

Correlations between ionized calcium, calcium ratio (ionized : total), total calcium and other blood constituents measured in 26 normal 21–28 day old lambs

Correlation between –	Correlation coefficient	Significance
Ionized calcium and total calcium	0,417	P < 0,05
Ionized calcium and inorganic phosphate	−0,127	NS
Ionized calcium and magnesium	−0,244	NS
Ionized calcium and total protein	0,096	NS
Calcium ratio and inorganic phosphate	−0,128	NS
Calcium ratio and magnesium	−0,279	NS
Calcium ratio and total protein	0,098	NS
Total calcium and inorganic phosphate	−0,046	NS
Total calcium and magnesium	−0,035	NS
Total calcium and total protein	0,028	NS

Correlation coefficient required for significance:
P < 0,05 = 0,388; P < 0,01 = 0,496 NS = Not significant

The levels of the nine blood constituents in the 26 suckling lambs are presented in Table 3 and the correlations between ionized calcium, the ratio between ionized calcium and total calcium, total calcium and the other blood constituents in Table 4.

Levels of the blood constituents (Tables 1 and 3)

The means (± S.D.) of the ionized calcium determinations in both the lactating ewes (4,25 ± 0,49 mg/100 ml) and suckling lambs (4,47 ± 0,65 mg/100 ml) are similar and also of the same order as that found for non-pregnant or early pregnant ewes (4,57 ± 0,44 mg/100 ml) (Belonje, 1973). Furthermore these values fall within the range of means (3,88 – 4,96 mg/100 ml) reported in a number of papers on ionized calcium levels in man (Hattner, Johnson, Bernstein, Wachman & Brackman, 1970; Reiss, Canterbury, Bercovitz & Kaplan, 1970; Hansen & Theodorsen, 1971; Li & Piechocki, 1971; Lindgärte & Zettervall, 1971; Pittinger, Chang & Faulkner, 1971; Raman, 1971; Schwartz, McConville & Christopherson, 1971).

The percentage of total calcium which is ionized in both lactating ewes (41,29 ± 4,4%) and suckling lambs (41,69 ± 5,69%) is similar and of the same order as that found in non-pregnant or early pregnant ewes (46,95 ± 4,36%) (Belonje, 1973). These values again fall within the range of 40,5 to 53,4% reported for humans (Moore, 1969; Sachs, Bourdeau & Balsan, 1969; Hattner *et al.*, 1970; Raman, 1970; Li & Piechocki, 1971; Raman, 1971).

The values for the other blood constituents which were determined fell within the normal ranges reported for sheep (Benjamin, 1961; Phillips, 1970; Simesen, 1970; Healy & Falk, 1974).

Correlations between the blood constituents (Tables 2 and 4)

The positive correlation between ionized calcium and total calcium is significant in both lactating ewes ($P < 0,05$) and suckling lambs ($P < 0,05$) but this is not of a sufficiently high order in both the ewes ($r = 0,416$) and lambs ($r = 0,417$) to be able to predict ionized calcium from total calcium levels. Similar findings have been reported for non-pregnant or early pregnant ewes ($P < 0,01$; $r = 0,418$) (Belonje, 1973) and humans (Hattner *et al.*, 1970; Schwartz *et al.*, 1971).

In the lactating ewes there was a significant negative correlation between ionized calcium and inorganic phosphate ($P < 0,01$), the ratio of ionized to total calcium and inorganic phosphate ($P < 0,01$) and total calcium and inorganic phosphate ($P < 0,05$). This reciprocal relationship between calcium and inorganic phosphate is well known (Hays & Swenson, 1970; Simesen, 1970) but it is of interest that in these ewes at least the magnitude of this relationship is greater between ionized calcium and inorganic phosphate ($r = -0,625$) then between total calcium and inorganic phosphate ($r = -0,388$) as it is ionized calcium which is the physiologically important fraction of total calcium. In the lambs, on the other hand, there was no significant correlation between inorganic phosphate and ionized calcium or total calcium or the ratio between the two. This may be because the level of inorganic phosphate is higher in young animals than in adults (Swenson, 1970; Healy & Falk, 1974) and that it is only when adult levels are reched that this reciprocal relationship becomes evident.

In the lactating ewes there was a significant positive correlation ($P < 0,01$; $r = 0,442$) between total calcium and magnesium. This was not found in the lambs or in non-pregnant or early pregnant ewes (Belonje, 1973). Whether this relationship holds true for lactating ewes in general is unknown.

No significant correlations were found in the ewes and the lambs between total calcium or ionized calcium or the ratio between the two and total plasma proteins.

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